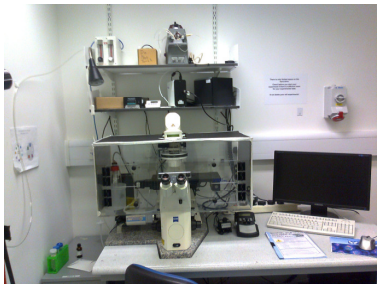
OPERATING INSTRUCTIONS



Timelapse-epi-inv (inverted epi-fluorescence) microscope

You must not operate this equipment without prior training from a BALM facility staff member.

To arrange training and for help please contact:

Facility Manager

Dr Ann Wheeler ext: 2406 a.p.wheeler@qmul.ac.uk

Microscopy Technologist

Isma Ali ext: 2407 i.ali@qmul.ac.uk

Standard Operating Procedure — Basic instructions

How to turn the equipment on:

1. Switch on the extension lead (A)
2. Computer and log in (B)
3. Camera – using the button on the camera. (C)
4. If you want to do bright field; bright field controller box (D)

How to turn the equipment off:

1. Bright field controller box (D)
2. Computer (B)
3. Extension lead switch (A)

If you are using CO2 the instructions for use of the Gas cylinder must be followed. See next to cylinder.

Rules of use:

This microscope should be treated with respect and care at all times.

This Microscope can only be used by Masters by Research or PhD students, Postdocs and members of staff. MBBS and BSc Students must be supervised by a member of research staff at all times.

The microscope lenses must be cleaned after every usage.

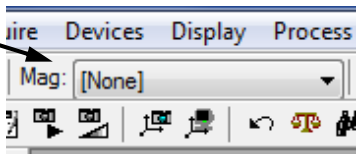
If you have any problems at all with the microscope, no matter how trivial they may seem please see a member of BALM facility staff immediately.

REMEMBER: You have 20GB of disk space on this microscope. Check before you start if you have room for your experiment. If not, delete your old data. Make sure you empty the recycle bin afterwards.

1) Open **MetaMorph** software

NB: The software takes a while to load

2) Place slide on microscope stage and select objective from the menu bar



Objectives available:

5x, 10x, 20x, 40x – air
40x, 63x – oil

Automated, do not move by hand

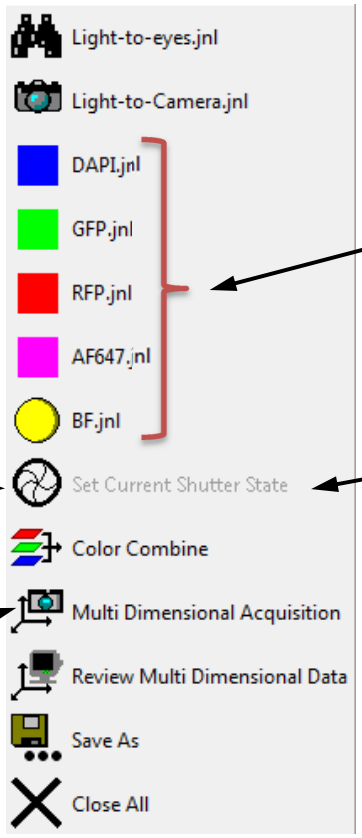
NB: microscope is inverted, so you will need to turn fixed specimens upside down

3) Select **Light to eyes** from toolbar on LHS

4) Select a channel

5) Check the shutter is open and find your sample

6) Select **Multi-Dimensional Acquisition** (a new window will appear)

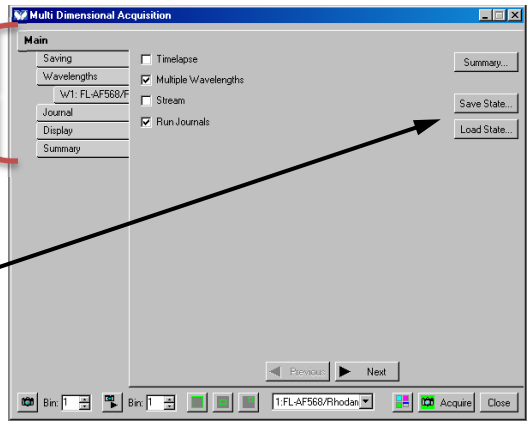


Use these buttons to change between the channels

Open and close shutter using **shutter state** button

7) Set the parameters of your experiment using the step by step tabs in the **multi-dimensional acquisition** window

8) Once you've set everything up you can save your acquisition settings using the **Save State** button and reload them using the **Load State** button

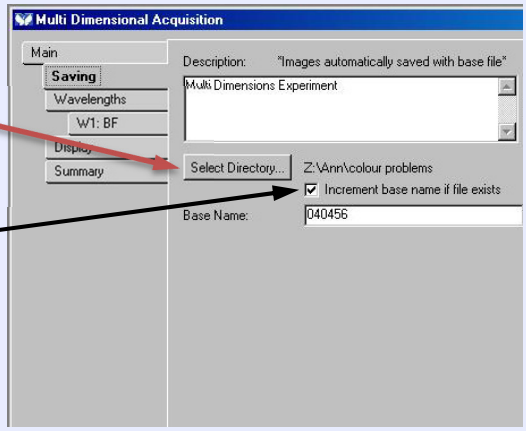


Save your data on the E-Drive

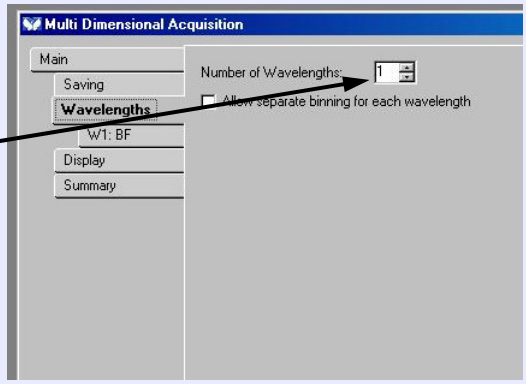
↘ In the **Saving** tab click **Select Directory** and choose your folder on the E-Drive

Type in a **Base Name** for your experiment

Check the **Incremental base name** box is ticked (this will automatically number your images under the base name you selected)



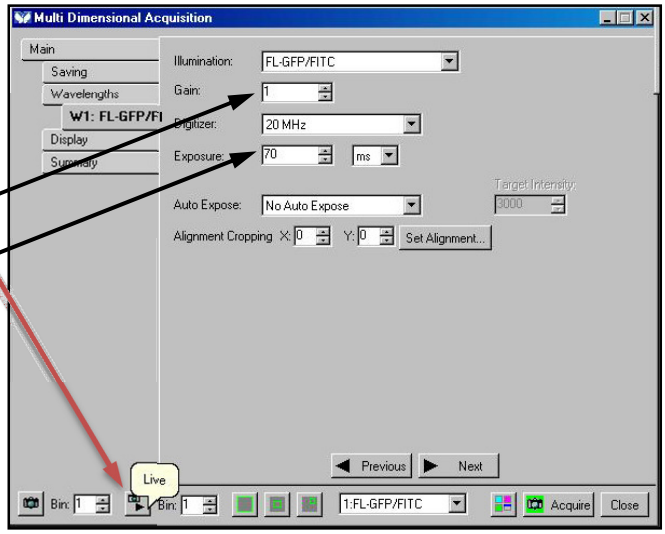
Choose the number of wavelengths you need



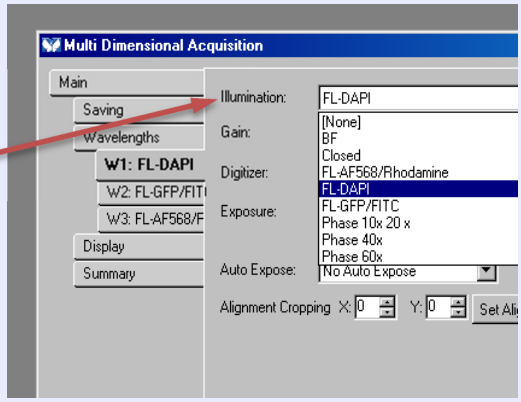
9) Select **Light to camera** from toolbar on LHS

10) Click **Show Live** icon
(live image will appear on screen,
you may need to adjust the **focus**)

11) Adjust **Gain** and **Exposure** for each wavelength



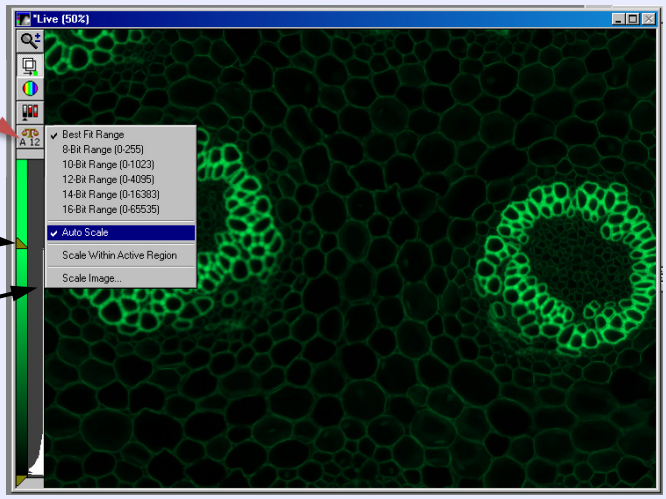
To change the assigned wavelengths select an illumination from the dropdown menu



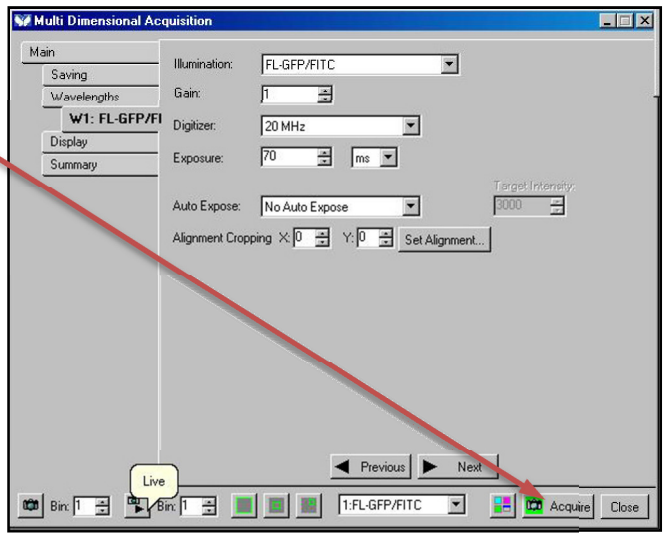
If you need to use **Auto Scale**, click on the icon on the LHS of the **Live Image Window**, and select it from the dropdown menu

Within the parameters of the gain and exposure that have been set, you can alter the image displayed by sliding the triangles on the side bar

NB. If the uppermost tail of the histogram is not viable then some areas of your image will be overexposed



12) Click **Acquire**
(a separate picture will be taken for each channel)



13) Creating a **Merged Image**

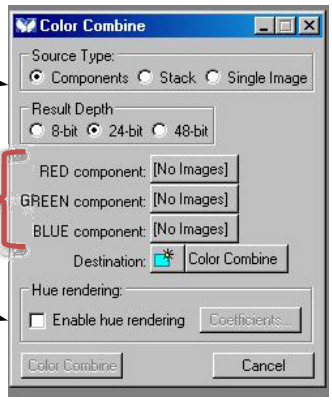
↘ click **Colour Combine** in toolbar on LHS

↘ select **48-bit** in **Colour Combine** window

↘ assign the appropriate images for each colour component

↘ click **Colour Combine**

↘ **Save** combined image to your folder in tiff format



When you have finished, transfer all your data to the **Z network drive**

PLEASE TIDY UP!!
Clean lenses, throw away used tissue/lens tissue, dispose of old slides in the yellow sharps bin